



#8

## **SEQUENCE LISTING**

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## <120> REDUCING IMMUNOGENICITIES OF IMMUNOGLOBULINS BY FRAMEWORK-PATCHING

<130> 655

**<140> US 09/892,613  
<141> 2001-06-27**

<160> 32

<170> PatentIn version 3.1

<210> 1  
<211> 369  
<212> DNA

<212> DNA  
<213> Artificial Sequence

<220>  
<223> FR-patched heavy chain variable region sequence (Full DNA Sequence) formed by joining the N- and C-terminal (SEQ 3 and 6) halves at the KpnI site.

<220>

<221> V\_region  
<222> (1) .. (369)  
<223>

<223>

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<400> 1
gaagtgcagc tgctggagtc tgggggaggc ttagtgtcagc ctggagggtc cctgaggctc      60
tcctgtgcag cctctggatt ctccctcagt atctatgaca tgtcttgggt tcgccaggca     120
ccgggaaagg ggctggagtg ggtgcatac attagtagtg gtggtggta cacctactat     180
ccagacactg tgaagggcccg attcaccatc tccagagaca atgccaagaa ctcccctgtac    240
ctgcaaatga acagtctgag ggtggaggac acagccttat attactgtgc aagacatagt    300
ggctacggta gtagctacgg ggttttgttt gcttactggg gccaaggac tctggtcact    360
gtctcttca                                         369
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<210> 2  
<211> 123  
<212> PRT  
<213> Chimaera sp.

<400> 2

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Phe Ser Ile Tyr  
20 25 30

Asp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
35 40 45

**Ala** Tyr Ile Ser Ser Gly Gly Gly Thr Thr Tyr Tyr Pro Asp Thr Val  
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr  
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Val Glu Asp Thr Ala Leu Tyr Tyr Cys  
85 90 95

Ala Arg His Ser Gly Tyr Gly Ser Ser Tyr Gly Val Leu Phe Ala Tyr  
 100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
 115 120

<210> 3  
<211> 111  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> N-template is a synthetic sense-strand oligonucleotide encoding a  
 amino acid 14-50 of the VH region (SEQ ID No. 2). The template is  
 PCR-amplified by two primers (SEQ ID No. 4 and 5)

<220>  
<221> V\_region  
<222> (1)..(111)  
<223>

<400> 3  
cctggagggt ccctgaggct ctcctgtgca gcctctggat ttccttcag tatctatgac 60  
atgtcttggg ttcgccaggc accggaaag gggctggagt gggtcgcata c 111

<210> 4  
<211> 57  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> 5' Primer is a synthetic sense-strand oligonucleotide encoding am  
 ino acid 1-19 of the VH region (SEQ ID No. 2). The 3' end of the  
 primer overlaps with the 5'end of the template by 18 nucleotides

<220>  
<221> primer\_bind  
<222> (1)..(57)  
<223>

<400> 4  
gaagtgcagc tgctggagtc tgggggaggc ttagtgtcagc ctggagggtc cctgagg 57

<210> 5  
<211> 48  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encodi  
 ng amino acid 43-59 of the VH region(SEQ ID No. 2). The primer o  
 verlaps with the template by 21 nucleotides.

<220>  
<221> primer\_bind  
<222> (1)..(48)  
<223>

<400> 5  
gttaggtggta ccaccaccac tactaatgta tgcgacccac tccagccc 48

<210> 6  
<211> 132  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> C-terminal is a synthetic sens -strand oligonucleotid encoding a

mino acid 68-111 of the VH region (SEQ ID No 2) The template is PCR-amplified by two primers (SEQ ID No 7 and 8)

<220>  
<221> V\_region  
<222> (1)..(132)  
<223>

<400> 6  
ttcaccatct ccagagacaa tgccaagaac tccctgtacc tgcaa atgaa cagtctgagg 60  
gtggaggaca cagcctata ttactgtgca agacatagtg gctacggtag tagtacggg 120  
gttttgttgc ct 132

<210> 7  
<211> 60  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> 5' Primer is a synthetic sense-strand oligonucleotide encoding amino acid 55-74 of the VH region (SEQ ID No 2). The 3' end of the primer overlaps with the 5' end of the template by 21 nucleotides

<220>  
<221> primer\_bind  
<222> (1)..(60)  
<223>

<400> 7  
ggtggtacca cctactatcc agacactgtg aagggccat tcaccatctc cagagacaat 60

<210> 8  
<211> 57  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 105-123 of the VH region (SEQ ID No 2). The primer and the template overlaps by 21 nucleotides.

<220>  
<221> primer\_bind  
<222> (1)..(57)  
<223>

<400> 8  
tgaagagaca gtgaccagag tcccttgcc ccagtaagca aacaaaaccc cgtagct 57

<210> 9  
<211> 321  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> FR-patched light chain variable region sequence formed by joining the N- and C-terminal (SEQ 11 and 14) halves at the KpnI site.

<220>  
<221> V\_region  
<222> (1)..(321)  
<223>

<400> 9  
gatatccaga tgacctcagtc tccatcctcc ctgtctgcct ctgtgggaga cagagtcacc 60  
attagttgca gggcaagtca ggacatttagc aattatcaa actggtatca gcagaaacca 120

ggtaaggctc cgaaactcct gatctactac actagtatata tacactcagg agtcccatca 180  
aggttcagtg gcagtggtc tggAACAGAA ttactctca ccattagctc cctgcagcca 240  
gaagattttg ccacttactt ttgccaacag ggtaatacgc ttccgtggac gttcgggtga 300  
ggcaccagg tggAAATCAA A 321

<210> 10  
<211> 107  
<212> PRT  
<213> Chimaera sp.

<400> 10

**Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly**  
1 5 10 15

**Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr**  
20 25 30

**Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile**  
**35 40 45**

Tyr Tyr Thr Ser Ile Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
65 70 75 80

Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp  
85 90 95

**Thr Phe Gly Gly Thr Lys Val Glu Ile Lys**  
**100 105**

<210> 11  
<211> 108  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> N-template is a synthetic sense-strand oligonucleotide encoding a  
mimic acid 11-46 of the VL region (SEQ ID No. 10). The template is  
PCR-amplified by two primers (SEQ ID No. 12 and 13)

<220>  
<221> V\_region  
<222> (1) .. (108)  
<223>

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<400> 11 ctgtctgcct ctgtggaga cagagtacc attagttgca gggcaagtca ggacattagc 60  
aattattaa actggtatca gcagaaaacca ggtaaggctc cgaaactc 108
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<210> 12  
<211> 51  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> 5' Primer is a synthetic sense-strand oligonucleotide encoding amino acid 1-17 of the VH region (SEQ ID No 10). The 3' end of the primer overlaps with the 5' end of the template by 21 nucleotides

<220>

<221> primer\_bind  
<222> (1)..(51)  
<223>

<400> 12  
gataatccaga tgacccagtc tccatectcc ctgtctgcct ctgtgggaga c

51

<210> 13  
<211> 40  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 40-53. The primer and the template overlaps by 18 nucleotides.

<220>  
<221> primer\_bind  
<222> (1)..(40)  
<223>

<400> 13  
ataatactagt gtagtagatc aggagttcg gagccttacc

40

<210> 14  
<211> 120  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> C-terminal is a synthetic sense-strand oligonucleotide encoding a amino acid 59-98 of the VH region (SEQ ID No 10) The template is PCR-amplified by tow primers (SEQ ID No 15 and 16)

<220>  
<221> V\_region  
<222> (1)..(120)  
<223>

<400> 14  
ccatcaaggc tcagtggcag tgggtctgga acagaattta ctctcaccat tagctccctg 60  
cagccagaag attttgccac ttactttgc caacagggtt atacgcttcc gtggacgttc 120

<210> 15  
<211> 49  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> 5' Primer is a synthetic sense-strand oligonucleotide encoding amino acid 50-65 of the VH region (SEQ ID No. 10). The 3' end of the primer overlaps with the 5'end of the template by 21 nucleotides

<220>  
<221> primer\_bind  
<222> (1)..(49)  
<223>

<400> 15  
ctacactagt atattacact caggagtccc atcaagggttc agtggcagt

49

<210> 16  
<211> 48  
<212> DNA  
<213> Artificial Sequence

<220>

<223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 92-107 of the VH region (SEQ ID No 10). The primer and the template overlaps by 21 nucleotides.

<220>  
 <221> primer\_bind  
 <222> (1)..(48)  
 <223>

<400> 16  
 tttgatttcc accttggtgc ctccaccgaa cgtccacgga agcgtatt 48

<210> 17  
 <211> 371  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> FR-patched heavy chain variable region sequence (Full DNA Sequence) formed by joining the N- and C-terminal (SEQ 19 and 22) halves at the KpnI site.

<220>  
 <221> V\_region  
 <222> (1)..(371)  
 <223>

<400> 17  
 caggtgcaac tggtgttc cggggcttag gtaaataagc ctggggcctc agtgaaggtc 60  
 tcctgcaagg cttctggcta cacatttacc agttacaata tgcactgggt acggcagcct 120  
 cctggaaagg gcctggaatg gattggagct atttatccag gaaatggtga tactagttac 180  
 aatcagaaat tcaagggcaa gcccacattg actgcagaca aatcctccag cacagcctac 240  
 atgcagctca gcagtctgac atctgaggac tctgcggctt attachgtgc aagatcgac 300  
 tacggtagta actacgtaga ctactttgac tactggggcc aaggcaccac tgttacagtc 360  
 tcctctgatc a 371

<210> 18  
 <211> 123  
 <212> PRT  
 <213> Chimaera sp.

<400> 18

Gln Val Gln Leu Val Ala Ser Gly Ala Glu Val Asn Lys Pro Gly Ala  
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr  
 20 25 30

Asn Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile  
 35 40 45

Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe  
 50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr  
 65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Ser His Tyr Gly Ser Asn Tyr Val Asp Tyr Phe Asp Tyr Trp

10Q

105

110

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Asp  
 115 120

<210> 19  
 <211> 114  
 <212> DNA  
 <213> Artificial Sequence  
 <220>  
 <223> N-template is a synthetic sense-strand oligonucleotide encoding a  
 amino acid 12-49 of the VH region (SEQ ID No. 18). The template i  
 s PCR-amplified by two primers (SEQ ID No. 20 and 21)  
 <220>  
 <221> V\_region  
 <222> (1)..(114)  
 <223>

<400> 19  
 aataaggcctg gggcctcagt gaaggtctcc tgcaaggcctt ctggctacac atttaccagt 60  
 tacaatatgc actgggtacg gcagcctcct ggaaggggcc tggaaatggat tgg 114

<210> 20  
 <211> 57  
 <212> DNA  
 <213> Artificial Sequence  
 <220>  
 <223> 5' Primer is a synthetic sense-strand oligonucleotide encoding am  
 ino acid 1-19 of the VH region (SEQ ID No 18). The 3' end of the  
 primer overlaps with the 5'end of the template by 24 nucleotides

<220>  
 <221> primer\_bind  
 <222> (1)..(57)  
 <223>

<400> 20  
 caggtgcaac tggtggcttc cggggctgag gtaaataagc ctggggcctc agtgaag 57

<210> 21  
 <211> 55  
 <212> DNA  
 <213> Artificial Sequence  
 <220>  
 <223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encodi  
 ng amino acid 43-60 of the VH region (SEQ ID No 18). The primer  
 and the template overlaps by 21 nucleotides.

<220>  
 <221> primer\_bind  
 <222> (1)..(55)  
 <223>

<400> 21  
 tgtaactagt atcaccattt cctggataaa tagctccaat ccattccagg cccct 55

<210> 22  
 <211> 126  
 <212> DNA  
 <213> Artificial Sequence  
 <220>  
 <223> C-terminal is a synthetic sense-strand oligonucleotide encoding a  
 mino acid 70-111 of the VH region (SEQ ID No 18) The template is

## PCR-amplified by tow primers (SEQ ID No 23 and 24)

<220>  
 <221> V\_region  
 <222> (1)..(126)  
 <223>

<400> 22  
 ttgactgcag acaaatcctc cagcacagcc tacatgcagc tcagcagtct gacatcttag 60  
 gactctgcgg tctattactg tgcaagatcg cactacggta gtaactacgt agactacttt 120  
 gactac 126

<210> 23  
 <211> 61  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> 5' Primer is a synthetic sense-strand oligonucleotide encoding amino acid 57-76 of the VH region (SEQ ID No 18). The 3' end of the primer overlaps with the 5'end of the template by 21 nucleotides.

<220>  
 <221> primer\_bind  
 <222> (1)..(61)  
 <223>

<400> 23  
 tgatactagt tacaatcaga aattcaaggg caaggccaca ttgactgcag acaaatcctc 60  
 c 61

<210> 24  
 <211> 59  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 105-123 of the VH region (SEQ ID No 18). The primer and the template overlaps by 21 nucleotides.

<220>  
 <221> primer\_bind  
 <222> (1)..(59)  
 <223>

<400> 24  
 tgatcagagg agactgtaac agtggtgccc tggcccccagt agtcaaagta gtctacgta 59

<210> 25  
 <211> 321  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> FR-patched light chain variable region sequence (Full DNA Sequence) formed by joining the N- and C-terminal (SEQ 27 and 30) halves at the BspEI site.

<220>  
 <221> V\_region  
 <222> (1)..(321)  
 <223>

<400> 25  
 gatattcaac tcacacagtc tccatcaagt ctttctgcat ctgtggggga cagagtcaca 60

attacttgca gggccagctc aagttaagt ttcatgcact ggtaccagca gaagccagga	120
tcctccccca aaccctggat ttatgccaca tccaacctgg cttccggagt ccctagtcgc	180
ttcagtggca gtgggtctgg gaccgagttc actctacaa tcagcagtt gcagcctgaa	240
gatttcgcca ctatattctg ccatcagtgg agtagtaacc cgctcacgtt cggtgctggg	300
accaagctga ccgttctacg g	321

<210> 26  
<211> 107  
<212> PRT  
<213> Chimaera sp.

<400> 26

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly	
1 5 10 15	

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Leu Ser Phe Met	
20 25 30	

His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr	
35 40 45	

Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser	
50 55 60	

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu	
65 70 75 80	

Asp Phe Ala Thr Tyr Phe Cys His Gln Trp Ser Ser Asn Pro Leu Thr	
85 90 95	

Phe Gly Ala Gly Thr Lys Leu Thr Val Leu Arg	
100 105	

<210> 27  
<211> 129  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> N-template is a synthetic sense-strand oligonucleotide encoding a  
minino acid 9-51 of the VL region (SEQ ID No. 26). The template is  
PCR-amplified by two primers (SEQ ID No. 28 and 29)

<220>  
<221> V\_region  
<222> (1)..(129)  
<223>

<400> 27	
tcaagtcttt ctgcatctgt gggggacaga gtcacaatta cttgcagggc cagctcaagt	60
ttaagttca tgcactggta ccagcagaag ccaggatcct ccccaaacc ctggatttat	120
gccacatcc	129

<210> 28  
<211> 45  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> 5' Primer is a synthetic sense-strand oligonucleotide encoding am  
ino acid 1-15 of the VH region (SEQ ID No 26). The 3' end of the

primer overlaps with the 5'end of the template by 21 nucleotides

<220>  
<221> primer\_bind  
<222> (1)..(45)  
<223>

<400> 28  
gatattcaac tcacacagtc tccatcaagt ctttctgcat ctgtg 45

<210> 29  
<211> 40  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 45-57. The primer and the template overlaps by 21 nucleotides.

<220>  
<221> primer\_bind  
<222> (1)..(40)  
<223>

<400> 29  
ggactccgga agccagggttg gatgtggcat aaatccaggg 40

<210> 30  
<211> 120  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> C-terminal is a synthetic sense-strand oligonucleotide encoding a amino acid 61-100 of the VH region (SEQ ID No 26). The template is PCR-amplified by tow primers (SEQ ID No 31 and 32)

<220>  
<221> V\_region  
<222> (1)..(120)  
<223>

<400> 30  
ttcagtggca gtgggtctgg gaccgagttc actctcacaa tcagcagttt gcagcctgaa 60  
gatttcgcca cttatttctg ccatcagtgg agtagtaacc cgctcacgtt cggtgctggg 120

<210> 31  
<211> 43  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> 5' Primer is a synthetic sense-strand oligonucleotide encoding amino acid 54-67 of the VH region (SEQ ID No 18). The 3' end of the primer overlaps with the 5'end of the template by 21 nucleotides.

<220>  
<221> primer\_bind  
<222> (1)..(43)  
<223>

<400> 31  
ggcttccgga gtccttagtc gtttcagttgg cagtgggtct ggg 43

<210> 32  
<211> 42

<212> DNA  
<213> Artificial Sequence

<220>  
<223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 94-107 of the VH region (SEQ ID No 26). The primer and the template overlaps by 21 nucleotides.

<220>  
<221> primer\_bind  
<222> (1)..(42)  
<223>

<400> 32  
ccgtagaacg gtcagcttgg tccccagcacc gaacgtgagc gg

42